"DESIGN AND SYNTHESIS OF SOME FLUORESCENT PROBES: STUDIES WITH BIOMACROMOLECULAR SYSTEMS"

Summary of Work (5000 Words)

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CONTENTS

Design and Synthesis of small molecules and their photophysical and biological interactions will be discussed in the thesis entitled "**DESIGN AND SYNTHESIS OF SOME FLUORESCENT PROBES: STUDIES WITH BIOMACROMOLECULAR SYSTEMS".** The thesis include following chapters.

Chapter I: *Design, Synthesis, and Biophysical Studies of Novel 1,2,3-Triazole-Based Compounds using Click reaction.*

Chapter IIA: *Design, Synthesis and photophysical studies of 4-azido coumarin with Proteins.*

Chapter IIB: *Interaction of 4-Azido coumarin with cyclodextrins and micelles.*

Chapter III: *Design, Synthesis, and Biophysical Studies of Thienopyridine derivatives.*

Chapter I

Design, Synthesis and Biophysical Studies of Novel 1,2,3-Triazole-Based Compounds using Click reaction

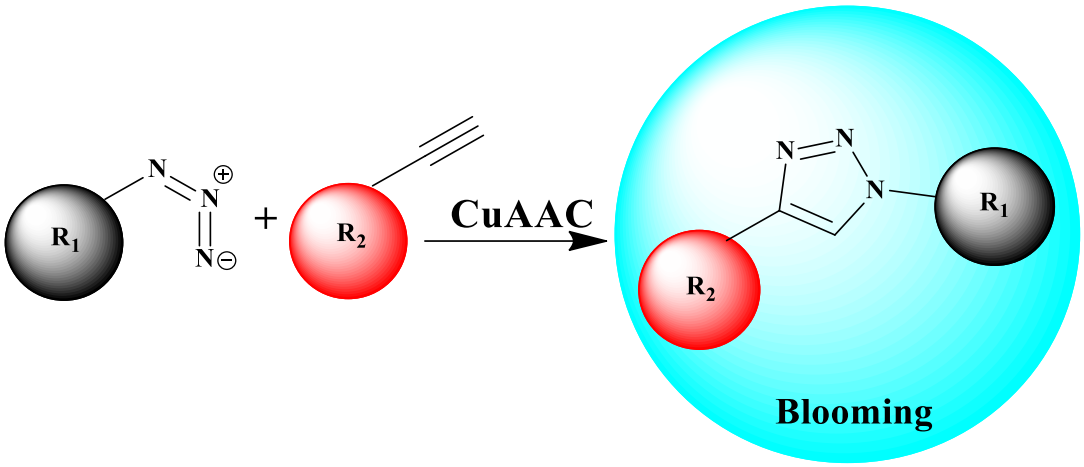
Introduction:

Heterocyclic chemistry is important in the area of research, because we cannot imagine our life without heterocyclic compounds. Heterocyclic compounds are important in drug development and discovery systems. Quinoline and coumarin are one of the important heterocyclic compounds in the field of medicinal chemistry research, with nitrogen and oxygen as hetero atom.

Quinoline and its derivatives are very important for their various medicinal applications such as anticancer [1,2], antimicrobial [3], antioxidant [4], antifungal [5] and anti tubercular agent [6,7]. Quinoline based heterocyclic compounds also show excellent anti HIV [8] and anti hypertensive [9] activities. Several quinoline derivatives show very good fluorescent property and those derivatives are used as fluorescent probe [10-12].

Coumarin and its derivatives are widely used for their excellent biological activities [13] such as anti-inflammatory [14], anti-cancer [15] and anti-bacterial activity [16]. Coumarin derivatives show very good fluorescent property, so, their derivatives are attractive starting materials for preparation of fluorescent probe.

Cu(I) catalysed alkyne-azide cyclo addition reaction (CuAAC reaction) or ‘Click Reaction’ is one of the important reaction for preparation of triazole derivatives via cyclo addition pathway. This reaction was first introduced by Sharpless [17,18]. Triazole based quinoline [19-22] and coumarin [14-16] compounds show promising medicinal activities and some of which are available drug today. Click reaction is capable of enhancing the fluorescent property of the resulting triazole compound (Scheme 1.1), which is one of the important features of this reaction [23].



Scheme 1.1: Schematic diagram of Click Reaction

Fluorescent probes are capable of showing bio sensing and bio imaging because of their high fluorescent intensity, excellent solubility, specificity and simple preparation [24]. Design, synthesis and development of fluorescent probe has great interest in biological and clinical research [24-29] because of their direct visualization and dynamic information [30]. Fluorescent probe can bind specifically with the receptor present on the cell, leading to sharp change in wavelength of the emitted light.

Interaction of compounds with BSA and HSA (serum albumins) was performed to find out the binding property of the compounds with serum albumins. The binding interaction with serum albumins (SA) is an important methodology to understand the medicinal property of the compound whether it can act as a drug or not at very preliminary stage of research [31-33].

Present work

Here in this chapter alkyne and azide derivatives have been designed for click reaction and then novel triazole based derivatives were synthesized (Fig. 1.1). The structure of the compounds were confirmed by NMR and mass spectroscopy and later on the biophysical studies of the synthesize compounds were carried out. Fluorescence study reveals that the microenvironment of SA, altered upon addition of the compounds.

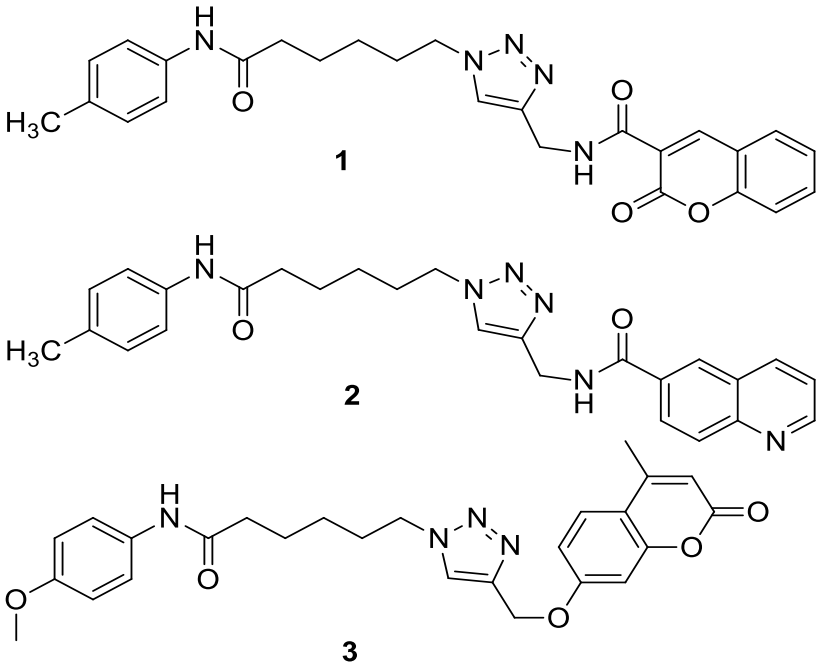
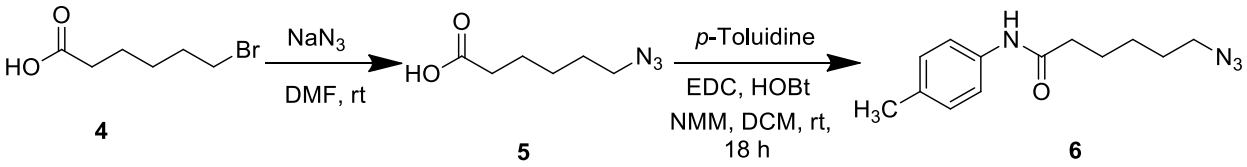


Figure 1.1. Triazole based derivatives, synthesized using Click reaction.

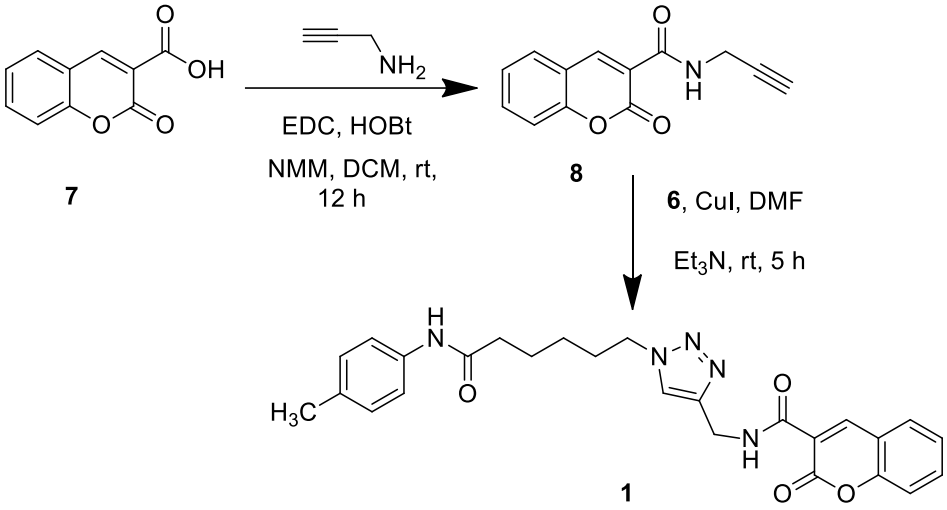
Result and Discussion:

Scheme 1.2: Methodology for the synthesis of compound 5 and 6



Procedure: 6-bromo hexanoic acid (**4**) treated with NaN_3, which afforded 6-azido hexanoic acid (**5**), further reaction of **5** with p-toluidine in presence of EDC, HOBt in dichloromethane (Scheme 1.2) gives compound **6**.

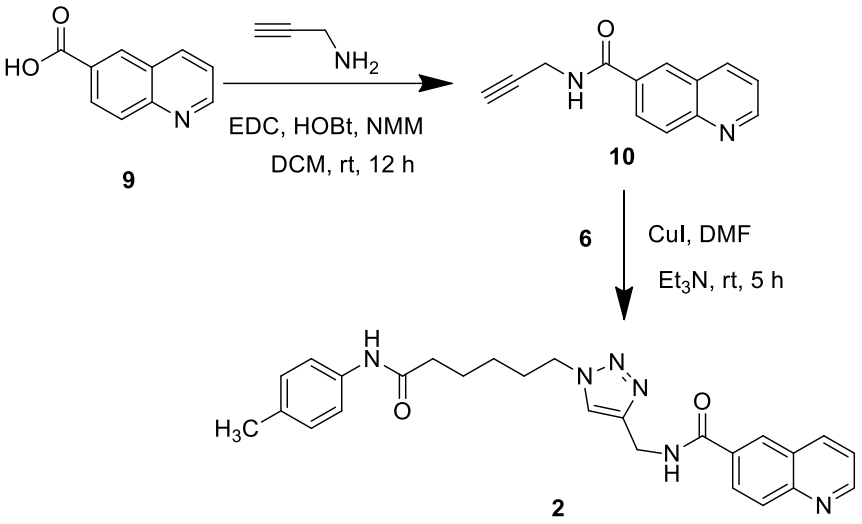
Scheme 1.3: Methodology for the synthesis of compound 8 and 1



Procedure: Coumarin-3-carboxylic acid was converted to its alkyne derivative **8** by coupling with propargyl amine in the presence of EDC, HOBt, and N-methylmorpholine (NMM) in dichloromethane (Scheme 1.3). Then CuAAC reaction was conducted between compound **8** and **6** in the presence of Cu(I) iodide in dimethyl formamide, which afforded triazole compound **1** (Scheme 1.3).

Characterisation data of compound **1**: **^{1}H NMR (400 MHz, $(CD_3)_2SO$)**, δ: 1.244-1.337 (m, 2H), 1.638-1.674 (m, 2H), 1.863-1.899 (m, 2H), 2.277 (s, 3H), 2.316 (t, 2H, *J* = 7.4 Hz), 4.395 (t, 2H, *J* = 7 Hz), 4.636 (d, 2H, *J* = 5.6 Hz), 7.11 (d, 2H, *J* = 8.4 Hz), 7.49 (dd, 1H, *J* = 8.2, 8.6 Hz), 7.564 (d, 1H, *J* =8.4 Hz), 7.574 (s, 1H), 7.804 (dd, 1H, *J* = 3.8, 4.6 Hz), 8.077 (s, 1H), 8.050 (d, 2H, *J* = 7.6 Hz), 8.954 (s, 1H), 9.177 (t, 1H, *J* = 5.2), 9.791 (s, 1H); **^{13}C NMR(400 MHz, $(CD_3)_2SO$)**, δ: 20.87, 24.98, 26.00, 30.02, 35.39, 36.55, 49.64, 116.61, 118.90, 119.20, 119.48, 123.25, 125.62, 129.45, 130.77, 132.22, 134.63, 137.25, 144.44,148.14, 154.36, 160.82, 161.54, 171.24; **MS-ESI (+)**: Calculated for $C_{26}H_{27}N_5O_4$ $[M+H]^+$ 474.210; found 474.1809.

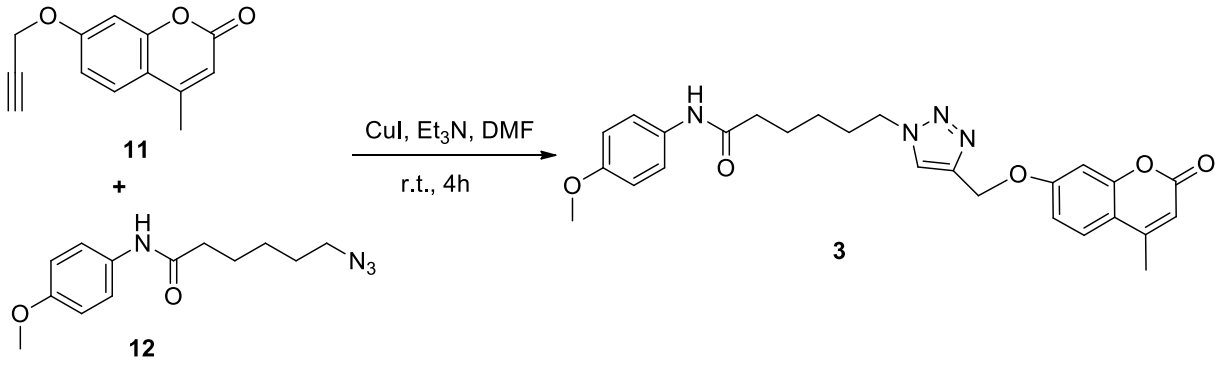
Scheme 1.4: Methodology for the synthesis of compound 10 and 2



Procedure: Quinoline alkyne (**10**) was synthesized from quinoline-6-carboxylic acid (**9**), shown in scheme 1.4, using the same procedure, described in scheme 1.3.

Characterisation data of compound **2**: **^{1}H NMR (400 MHz, $(CD_3)_2SO$)**, δ: 1.292-1.346 (m, 2H), 1.642-1.678 (m, 2H), 1.869-1.906 (m, 2H), 2.245 (s, 3H), 2.320 (t, 2H, *J* = 7.6 Hz), 4.395 (t, 2H, *J* = 7.2 Hz), 4.36 (t, 2H, *J* = 5.6 Hz), 7.113 (d, 2H, *J* = 8 Hz), 7.49 (d, 2H, *J* = 8 Hz), 7.666 (dd, 1H, *J* = 4.4, 4.0 Hz), 8.088 (s, 1H), 8.142 (d, 1H, *J* = 8.8 Hz), 8.257 (d, 1H, *J* = 2 Hz), 8.524 (d, 1H, *J* = 8 Hz), 8.607 (d, 1H, *J* = 1.6 Hz), 9.042 (t, 1H, *J* = 4 Hz), 9.327 (d, 1H, *J* =5.6 Hz), 9.816 (s, 1H); **^{13}C NMR(400 MHz, $(CD_3)_2SO$)**, δ: 20.87, 24.99, 26.03, 30.07, 35.50, 36.57, 49.61, 119.50, 122.64, 123.36, 127.53, 128.21, 128.56, 129.45, 132.23, 132.43, 137.25, 137.57, 145.27, 149.13, 152.54, 166.13, 171.24; **MS-ESI (+)**: Calculated for $C_{26}H_{28}N_6O_2$ $[M+H]^+$ 457.23; found 457.2005.

Scheme 1.5: Methodology for the synthesis of compound 12 and 3



Procedure: Compound **11** was synthesized using the literature procedure [34]. Compound **12**, was synthesized using the methodology described in Scheme 1.2 (only difference is the use of p-Anisidine instead of p-Toluidine). Compound **3** was obtained by the 'Click Reaction' between the compound **11** and **12** in the presence of Cu(I) iodide as catalyst in dimethyl formamide (DMF) medium (Scheme 1.5).

Characterisation data of compound **3**: **^{1}H NMR (400 MHZ, $CDCl_3$),** δ: 1.245-1.322 (2H, m), 1.641-1.697 (2H, m), 1.849-1.92 (2H, m), 2.257 (2H, t, J = 7 Hz), 2.325 (3H, s), 3.702 (3H, s), 4.318 (2H, t, J = 6.8 Hz), 5.17 (2H, s), 6.074 (1H, s), 6.893 (1H, d, J = 2Hz), 6.871 (1H, d, J = 2 Hz), 6.776 (1H, s),6.754 (1H, d, J = 8.8 Hz), 7.487 (1H, s), 7.376 (2H, d, J = 8.8 Hz), 7.447 (1H, d, J = 8.8 Hz), 7.588 (1H, s). **^{13}C NMR (400 MHZ, $CDCl_3$),** δ: 18.71, 24.58, 25.92, 29.92, 36.84, 50.15, 55.47, 62.34, 102.01, 112.18, 112.95, 114.03, 114.08, 121.63, 123.10, 125.75, 131.16, 137.10, 142.89, 152.76, 156.28, 161.16, 161.37, 170.71. **MS-ESI (+)**:, m/z: 477.2136 [M+H]+. $C_{26}H_{28}N_4O_5$. Calculated, m/z: 477.21.

Steady-state absorption and emission studies at 298K:

Interaction of the compounds **1**, **2** and **3** with BSA/HSA has been investigated by steady state absorption spectra (Fig. 1.2 and 1.3). Absorption spectra of free SA has been changed upon addition of compound 1, 2 and 3. The result implies interaction of protein with the compounds and alteration in the microenvironment of SA.

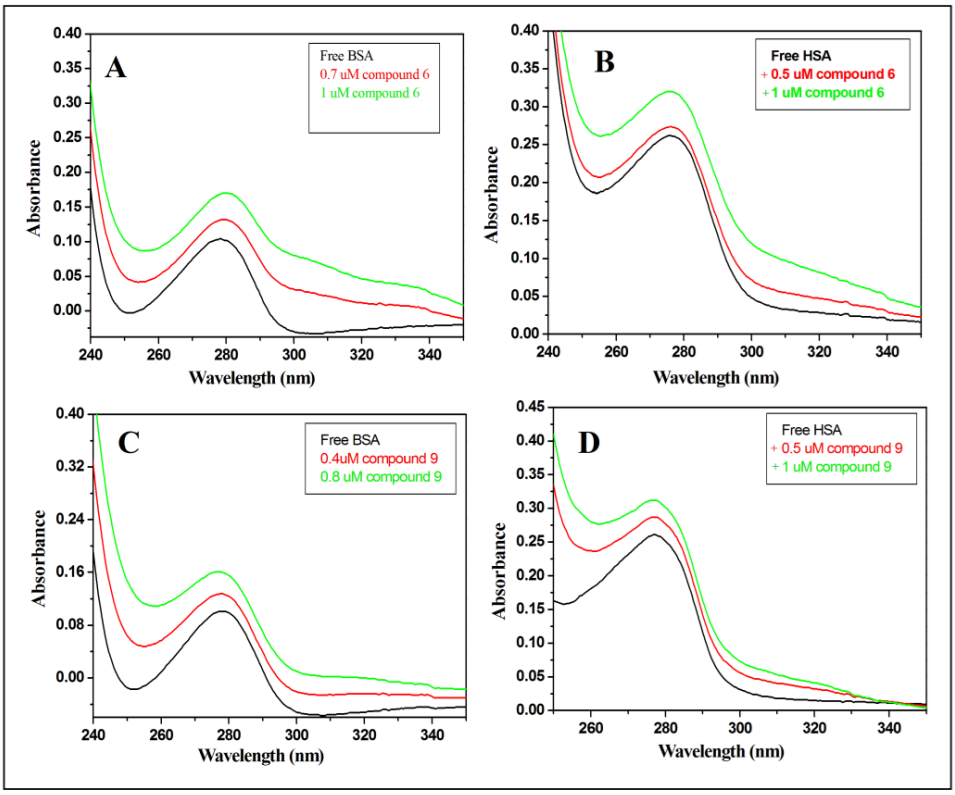


Figure 1.2. Absorption spectra of (**A**) and (**C**) Free BSA (5 µM) and varying concentration of compound **1** and **2** respectively (**B**) and (**D**) Free HSA (5 µM) and varying concentration of compound **1** and **2** respectively, in aqueous phosphate buffer (pH 7) at 298K.

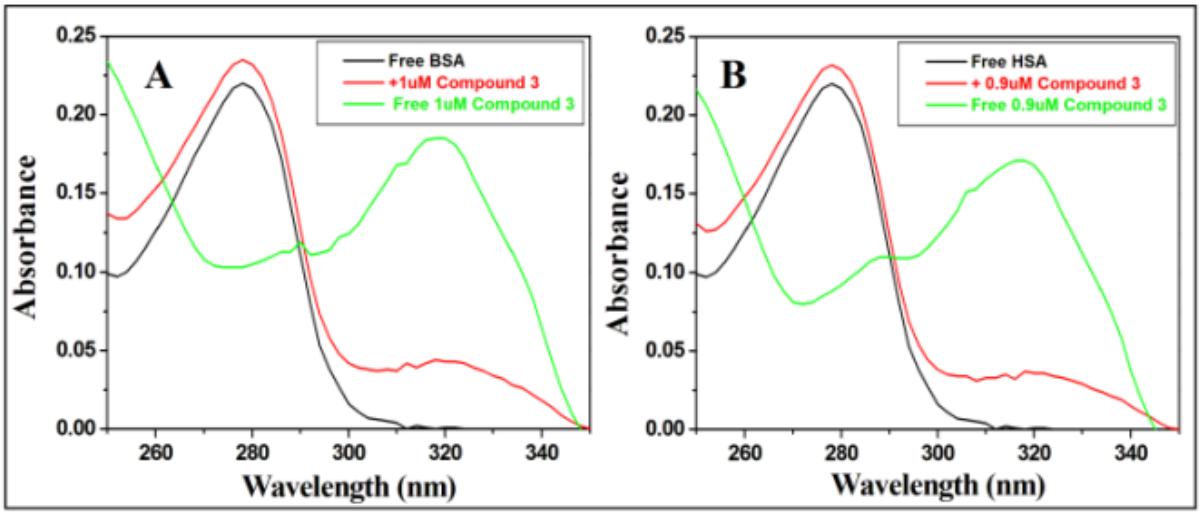


Figure 1.3. (**A**) Absorption spectra of 5 µM of BSA with varying concentration of compound **3** (**B**) Absorption spectra of 5 µM of HSA with varying concentration of compound **3**, in aqueous phosphate buffer (pH 7) at 298 K.

The fluorescence emission of emitting Trp (s) residues in SA are gradually quenched and blue shifted upon addition of compound **1**, **2** and red shifted upon addition of compound **3** (Fig. 1.4 and 1.5). The quenching and the shift of emission maxima implies that the emitting Trp (s) residues experiencing different environment when interact with the compounds. This is an important indication of protein ligand interaction.

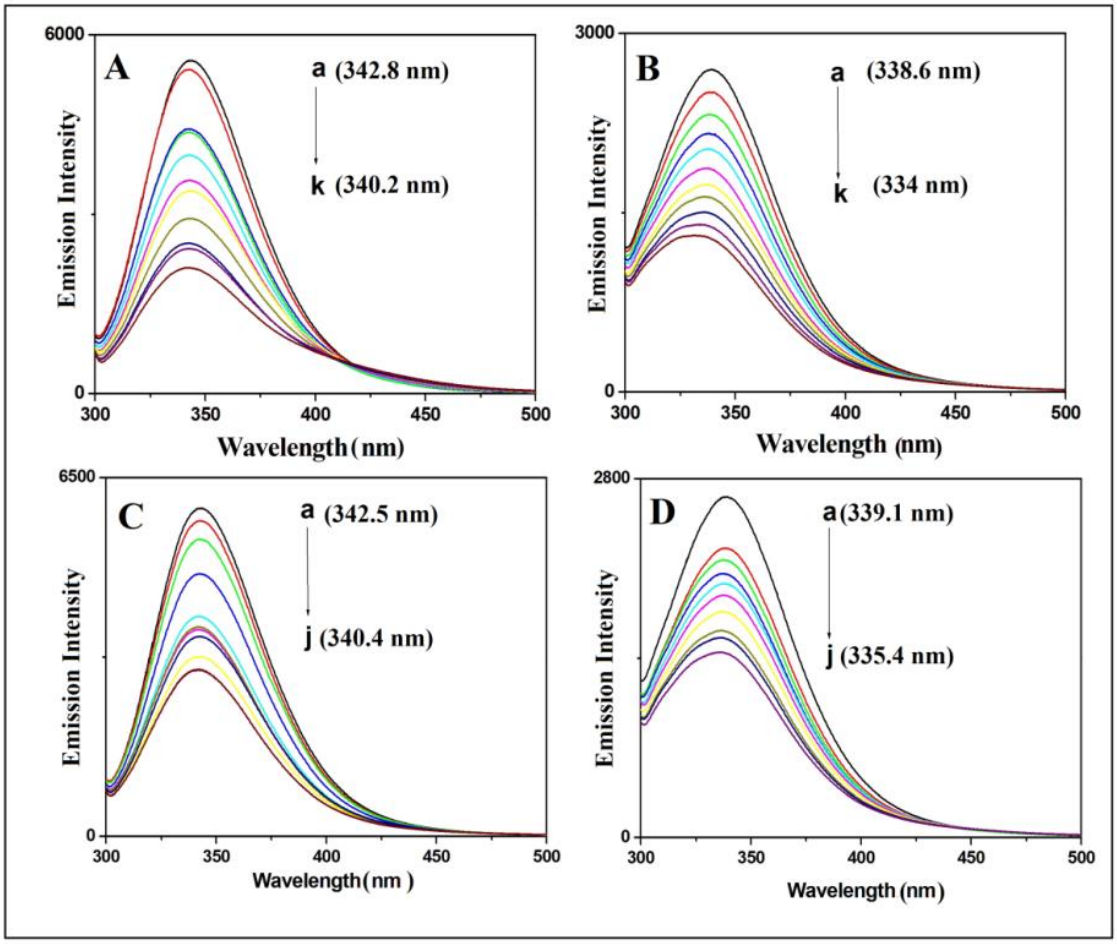


Figure 1.4. Fluorescence emission spectra of (**A**) and (**C**) Free BSA (5 µM) and varying concentration of compound **1** and **2** respectively (**B**) and (**D**) Free HSA (5 µM) and varying concentration of compound **1** and **2** respectively, in aqueous phosphate buffer (pH 7) at 298K.

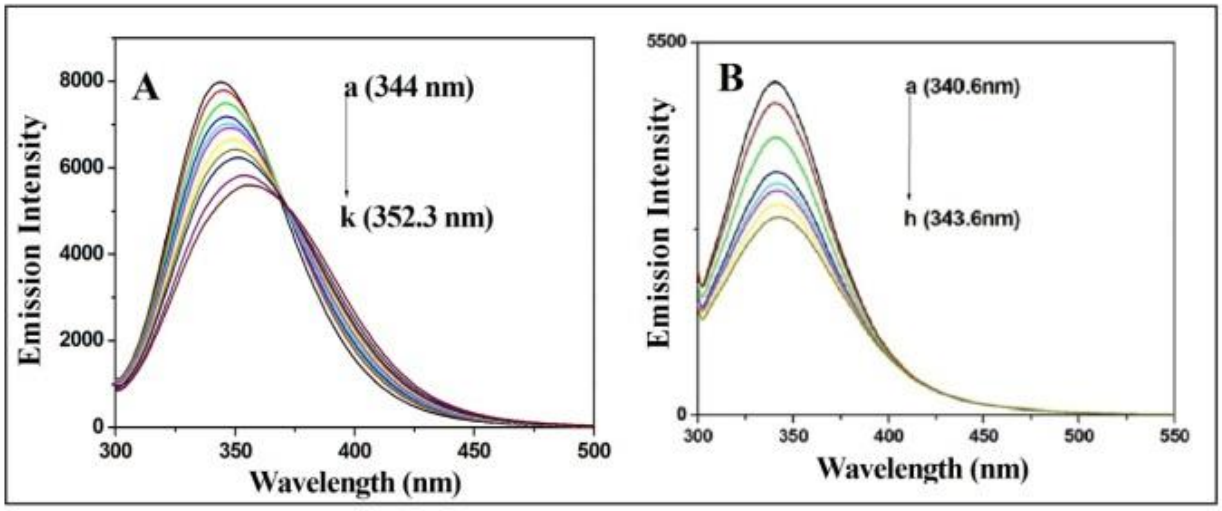


Figure 1.5. Fluorescence emission spectra of **(A)** 5 μM of BSA with varying concentration of compound**3 (B)** 5 μM of HSA with varying concentration of compound **3**, in aqueous phosphate buffer (pH 7) at 298 K.

Table 1.1. Binding constant values of the complex of compound 1, compound 2 and compound 3 with both the serum albumins in aqueous buffer (pH 7) at 298 K.

System	K_{SV} (M^{-1})	K_b (M^{-1})
BSA- compound **1**	9.36×10^5	11.5×10^5
BSA- compound **2**	3.52×10^5	11.0×10^5
HSA- compound **1**	8.08×10^5	8.91×10^5
HSA- compound **2**	6.65×10^5	5.56×10^5
BSA-compound **3**	1.23×10^5	1.26×10^5
HSA-compound **3**	1.88×10^5	2.11×10^5

Conclusion:

Novel heterocyclic coumarin and quinoline derivatives were synthesized using click reaction and its interactions were carried out with BSA and HSA by steady state absorption and fluorescence studies at 298 K. The photophysics of these interactions reveal the stronger binding ability of the compound (K_b is 10^5 orders of magnitude, Table 1.1) by fluorescence quenching of Trps with both the serum albumins. The binding of the compounds perturb the microenvironment of the proteins.

References:

1) W. A. Denny, W. R. Wilson, D. C. Ware, G. J. Atwell, J. B. Milbank and R. J. Stevenson, US7064117B2, 2006.
2) Z.-B. Shi, L. Zhang, Z.-Y. Bin, X.-R. Cao, Z.-N. Gong and J.-X. Li, *Lett. Drug Des. Discovery*, 2013, **10**, 420.
3) H. G. Bhatt, Y. K. Agrawal and M. J. Patel, *Med. Chem. Res*., 2015, **24**, 1662.
4) G. X. Li, Z. Q. Liu and X. Y. Luo, *Eur. J. Med. Chem*., 2010, **45**, 1821.
5) A. H. Kategaonkar, R. U. Pokalwar, S. S. Sonar, V. U. Gawali, B. B. Shingate and M. S. Shingare, *Eur. J. Med. Chem*., 2010, **45**, 1128.
6) K. D. Thomas, V. Adhikari, S. Telkar, I. H. Chowdhury, R. Mahmoode, N. K. Pal, G. Rowd and E. Sumesh, *Eur. J. Med. Chem*., 2011, **46**, 5283.
7) S. Eswaran, A. V. Adhikari and R. A. Kumar, *Eur. J. Med. Chem*., 2010, **45**, 957.
8) L. Strekowski, J. L. Mokrosz, V. A. Honkan, A. Czarny, M. T. Cegla, S. E. Patterson, R. L. Wydra and R. F. Schinazi, *J. Med. Chem*., 1991, **34**, 1739.
9) N. Muruganantham, R. Sivakumar, N. Anbalagan, V. Gunasekaran and J. T. Leonard, *Biol. Pharm. Bull*., 2004, **27**, 1683.
10) G. Li, D. Zhu, L. Xue and H. Jiang, *Org. Lett*., 2013, **15**, 5020.
11) Z. Mao, L. Hu, X. Dong, C. Zhong, B. F. Liu and Z. Liu, *Anal. Chem*., 2014, **86**, 6548.
12) H. L. Lu, W. K. Wang, X. X. Tan, X. F. Luo, M. L. Zhang, M. Zhang and S. Q. Zang, *Dalton Trans*., 2016, **45**, 8174.
13) M. A. Musa, J. S. Cooperwood and M. O. F. Khan, *Curr. Med. Chem*., 2008, **15**, 2664.
14) H. A. Stefani, K. Gueogjan, F. Manarin, S. H. Farsky, J. Z. Schpector, I. Caracelli, S. R. P. Rodrigues, M. N. Muscara, S. A. Teixeira and J. R. Santin, *Eur. J. Med. Chem*., 2012, **58**, 117.
15) W. J. Zhang, Z. Li, M. Zhou, F. Wu, X. Y. Hou, H. Luo, H. Liu, X. Han, G. Y. Yan and Z. Y. Ding, *Bioorg. Med. Chem. Lett*., 2014, **24**, 799.
16) Y. A. Shi and C. H. Zhou, *Bioorg. Med. Chem. Lett*., 2011, **21**, 956.
17) C. D. Hein, X. M. Liu and D. Wang, *Pharm. Res*., 2008, **25**, 2216.
18) H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed*., 2001, **40**, 2004.
19) K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill and Q. A. Wang, *Org. Lett*., 2004, **4**, 4603.

20) Y. A. Shi and C. H. Zhou, *Bioorg. Med. Chem. Lett*., 2011, **21**, 956.

21) H. A. Stefani, K. Gueogjan, F. Manarin, S. H. Farsky, J. Z. Schpector, I. Caracelli, S. R. P. Rodrigues, M. N. Muscara, S. A. Teixeira and J. R. Santin, *Eur. J. Med. Chem*., 2012, **58**, 117.

22) W. Zhang, Z. Li, M. Zhou, F. Wu, X. Hou, H. Luo, H. Liu, X. Han, G. Yan, Z. Ding and R. Li, *Bioorg. Med. Chem. Lett*., 2014, **24**, 799.

23) P. Thasnima and D. Bahulayan, *New J. Chem.*, 2017,**41**, 13483.

24) H. Zhu, J. Fan, J. Du and X. Peng, *Acc. Chem. Res*., 2016, **49**, 2115.

25) H. Kobayashi, M. Ogawa, R. Alford, P. L. Choyke and Y. Urano, *Chem Rev*., 2010, **110**, 2620.

26) Y. Urano, D. Asanuma, Y. Hama, Y. Koyama, T. Barrett, M. Kamiya, T. Nagano, T. Watanabe, A. Hasegawa, P. L. Choyke and H. Kobayashi, *Nature Medicine*, 2008, **15**, 104.

27) J. Li, C.F. Zhang, S.H. Yang, W.C. Yang and G.F. Yang, *Anal. Chem*., 2014, **86**, 3037.

28) Y. Liu, M. Han, H.Y. Zhang, L. X. Yang and W. Jiang, *Org. Lett*., 2008, **10**, 2873.

29) M. Zhang, M. Yu, F.Li, M. Zhu, M. Li, Y. Gao, L. Li, Z. Liu, J. Zhang, D. Zhang, T. Yi and C. Huang, *J. Am. Chem. Soc*., 2007, **129**, 10322.

30) T. Ueno and T. Nagano, *Nat. Methods*, 2011, **8**, 642.

31) S. Paul, N. Sepay, S. Sarkar, P. Roy, S. Dasgupta, P. S. Sardar and A. Majhi, *New J. Chem*., 2017, **41**, 15392.

32) S. Paul, P. Roy, P. S. Sardar and A. Majhi, *ACS Omega*, 2019, **4**, 7213.

33) S. Paul, R. Ghanti, P. S. Sardar and A. Majhi, *Chem. Het Com.*, 2019, **55**, 607.

34) M. Zayane, A. Rahmouni, M. D. Remadi, M. B. Mansour, A. Romdhane and H. J. B. Jannet, *Enzyme Inhib. Med. Chem*., 2016, **31**, 1566.

Publications:

[1] **Sandip Paul**, Pritam Roy, Pinki Saha Sardar and Anjoy Majhi*, **ACS Omega, 2019,** 4, 7213.

[2] **Sandip Paul**, Raju Ghanti, Pinki Saha Sardar and Anjoy Majhi*, **Chemistry of Heterocyclic Compounds, 2019,** 55(7), 607.

Oral presentation:

[1] **International Symposium on Recent Advances in Chemistry and Material Sciences (2019):** Design and synthesis of fluorescent probe based on HDAC scaffold and its biophysical studies; **Sandip Paul** and Anjoy Majhi.

Poster presentation:

[1] **International seminar on 'ITINERARY OF CLASSICAL TO MODERN ERA OF CHEMISTRY' (2019):** Synthesis of triazole based derivatives using click reaction and their interaction with serum albumins; **Sandip Paul** and Anjoy Majhi.

Chapter II

Introduction:

Serum albumins (SA) such as BSA and HSA are the most abundant soluble plasma proteins which play key role in many biological processes [1,2]. The molecular weight of BSA and HSA are 66 and 66.5 KDa respectively and both the proteins show approximately 80% sequence similarity. Crystal structure reveals that 582 and 585 amino acid residues present in BSA and HSA respectively. Two tryptophan (Trp) residues (position 213 and 134) are present in BSA and HSA contain only one Trp residue located at 214. Protein ligand binding interaction is an important method in medicinal chemistry research at preliminary stage [3, 4].

Chapter II A

Design, Synthesis and photophysical studies of 4-azido coumarin with Proteins

Present work:

Present chapter discuss the design and synthesis of 4-Azido coumarin (4-AC, Fig. 2.1) [5, 6], one of the parent compound of click reaction (Scheme 1.1). We design a coumarin azide moiety and later on photophysical studies have been carried out with proteins to understand the binding phenomenon of azide moiety. Interaction of 4-AC with proteins reveals that the microenvironment of the protein is been altered.

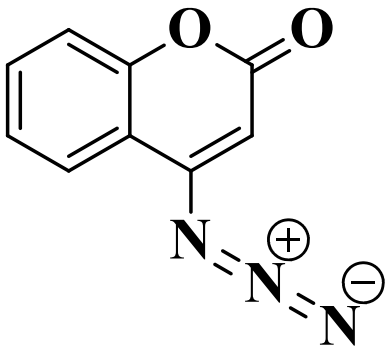
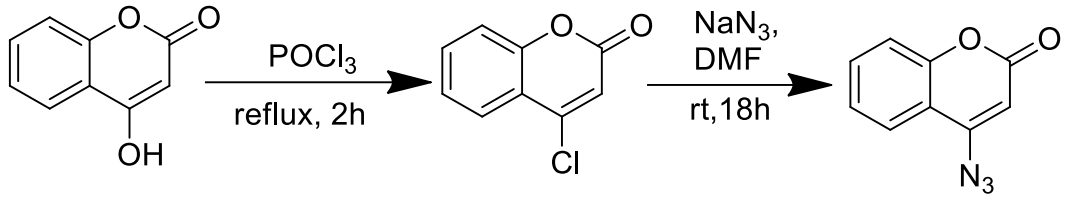


Figure 2.1: 4-Azidocoumarin or 4-azido-2H-chromen-2-one (4-AC)

Result and Discussion:

Scheme 2.1: Methodology for the synthesis of 4-Azido coumarin



4-Azido Coumarin

Procedure: 4-Hydroxy coumarin treated with $POCl_3$ under reflux condition for two hours to afford 4-Chloro coumarin, which on treatment with NaN_3 in DMF affords 4-Azido coumarin [5,6] (Scheme 2.1). Characterization data matches well with the literature values.

Steady-state fluorescence studies monitoring tryptophan emission of the BSA/HSA at 298 K:

The fluorescence spectra of free BSA and free HSA is gradually quenched and blue shifted from 343 nm (in free BSA) to 339 nm and 338.6 nm (in free HSA) to 334.2 nm upon gradual addition of 4-AC at 298K in aqueous phosphate buffer of pH7 (Fig. 2.2). This implies that the SA in complex with 4-AC is in more hydrophobic environment compare to free SA (Table 2.1, Fig. 2.3 and 2.4).

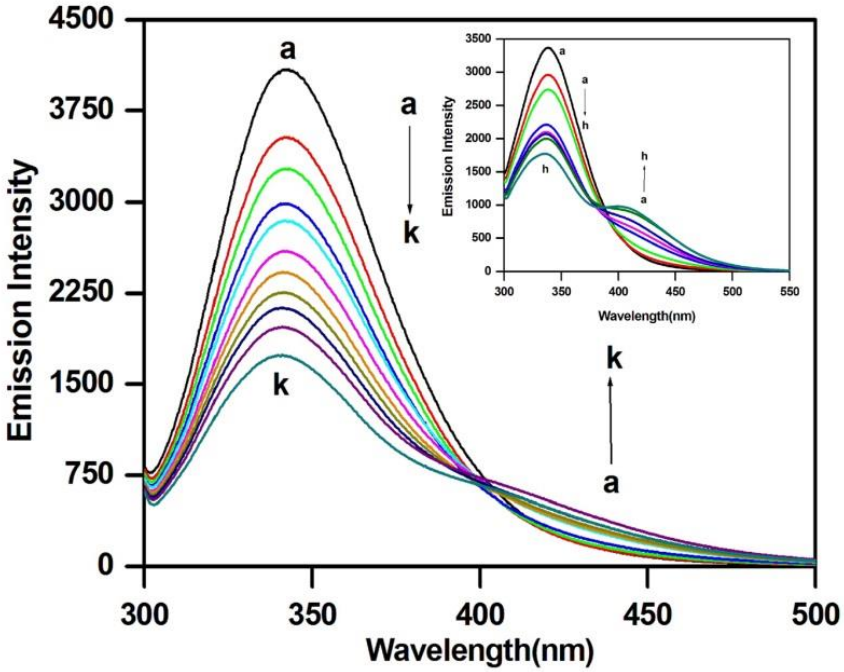


Figure 2.2. Fluorescence spectra of BSA at 298 K with varying concentration of 4-AC, *Inset:* Fluorescence spectra of HSA at 298 K with varying concentration of 4-AC.

Table 2.1. Binding Constants and thermodynamic parameters of binding in serum albumin-4-AC complexes:

System	Binding Constants (M^{-1})	Binding Site (n)	ΔG^0 (Cal mol^{-1})	ΔH^0 (Cal mol^{-1})	ΔS^0 (Cal K^{-1} mol^{-1})
BSA-4-AC	$0.96x10^6$ at 298 K $0.88 x10^6$ at 308 K	1	$-8.16 x10^3$ (-34.14 kJ mol^{-1})	$-1.59x10^3$ (-6.65 kJ mol^{-1})	22.05 (92.21 J mol^{-1})
HSA-4-AC	$0.57x10^6$ at 298 K $3.20 x10^6$ at 308 K	1	$-7.85 x10^3$ (-32.84 kJ mol^{-1})	$31.46 x10^3$ (131.63 kJ mol^{-1})	131.91 (551.91 J mol^{-1})

Fluorescence quenching studies in serum albumin-4-AC complexes:

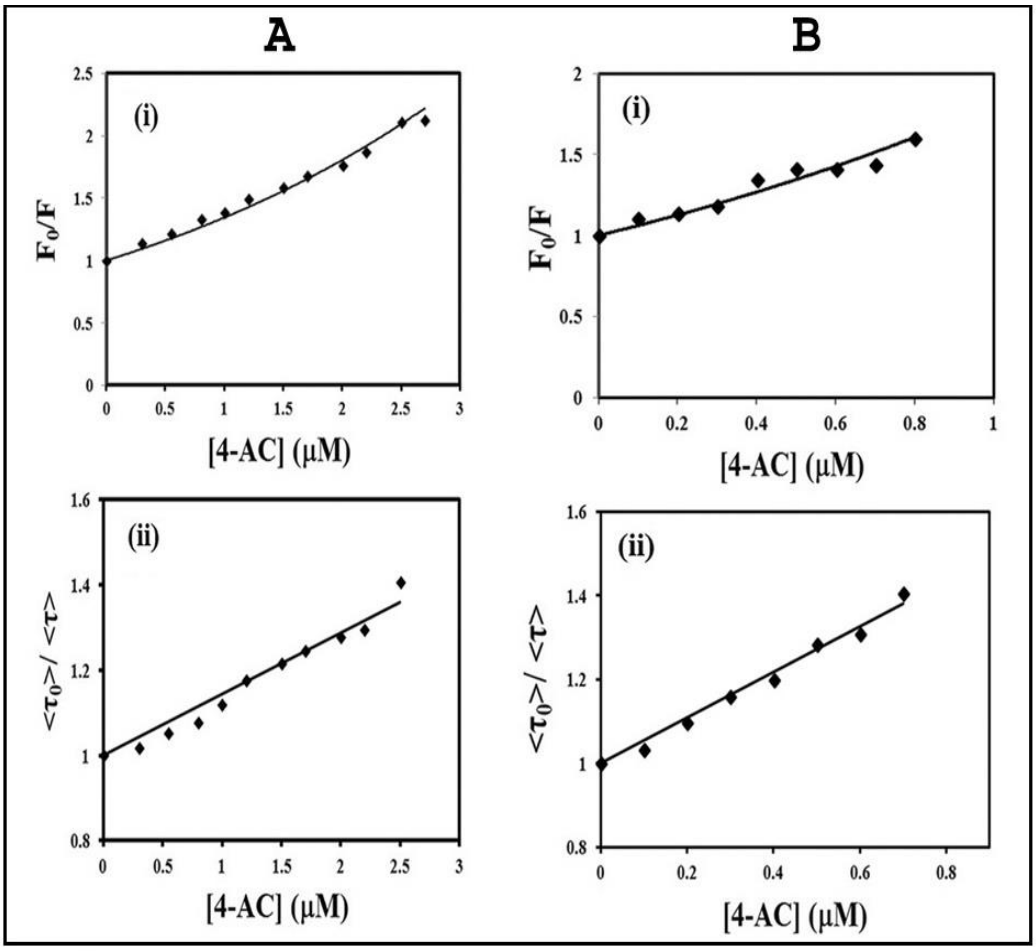


Figure 2.3. (A) & (B)-(i) Plot of F_0/F against [4-AC] in BSA and HSA respectively**,**(A) & (B)-(ii)plot of $<\tau_0>/<\tau>$ against [4-AC] in BSA and HSA respectively.

Fluorescence lifetime measurement of serum albumins and 4-AC complexes:

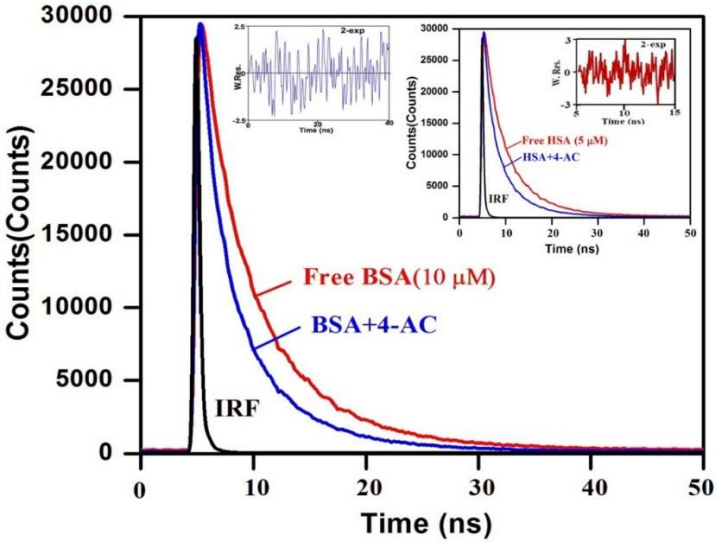


Figure 2.4. Fluorescence decay of free BSA, complex of BSA with 4-AC, *Inset:* Fluorescence decay of free HSA, complex of HSA with 4-AC.

Circular dichroism studies:

Circular dichroism spectroscopy was performed to find out the change in secondary structure of proteins upon addition of 4-AC. In CD spectroscopy two negative bands appear at 208 and 222 nm, and the change is characterised by α-helical content in proteins. [7-9]. In case of BSA, α-helical content is slightly changed from 63% to 62%, where as in case of HSA α-helical content change from 52% to 49% (Fig. 2.5). Hence the structural perturbation is observed in SA due to binding with 4-AC.

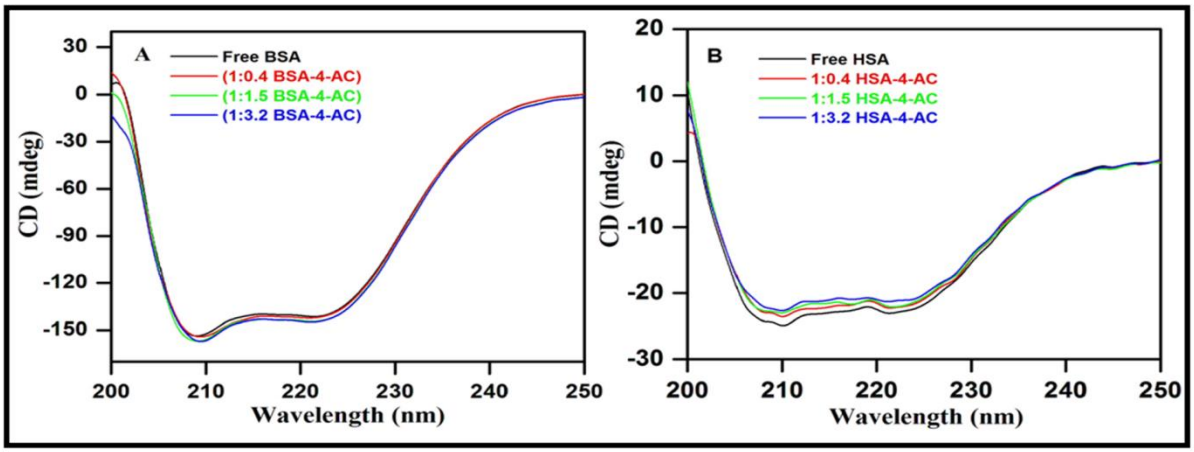


Figure 2.5. Circular dichroism (CD) spectra of (**A**) BSA and (**B**) HSA with 4-AC in different molar ratios of protein and ligand at 298 K in phosphate buffer of pH 7.

Phosphorescence spectra of Trp residues in the serum albumin-4-AC complexes at 77K:

Phosphorescence measurement was performed at 77 K to understand the perturbation of Trp residues (Trp 134 and Trp 213 in BSA and Trp 214 in HSA) in the SA–ligand complexes. The phosphorescence spectra of free SA and its complex with 4-AC was carried out in 40% ethylene glycol at 77 K. Free BSA exhibits a distinct (0,0) band at ~413 nm with a small shoulder at ~405.0 nm correspond to Trp 213 and Trp 134 respectively (Fig. 2.6, Table 2.2). The phosphorescence spectra are quenched compared to that of free proteins in both the complexes. The bandwidths (obtained using a curve-fitting procedure) [10, 11] of the (0,0) band in the different systems are calculated and it is decreased from free SA. Decrease in phosphorescence spectra and bandwidth indicate that that the micro environments of Trp residues in SA (Trp213 for BSA and Trp214 for HSA) are perturbed compared to that of the free proteins (Table 2.2).

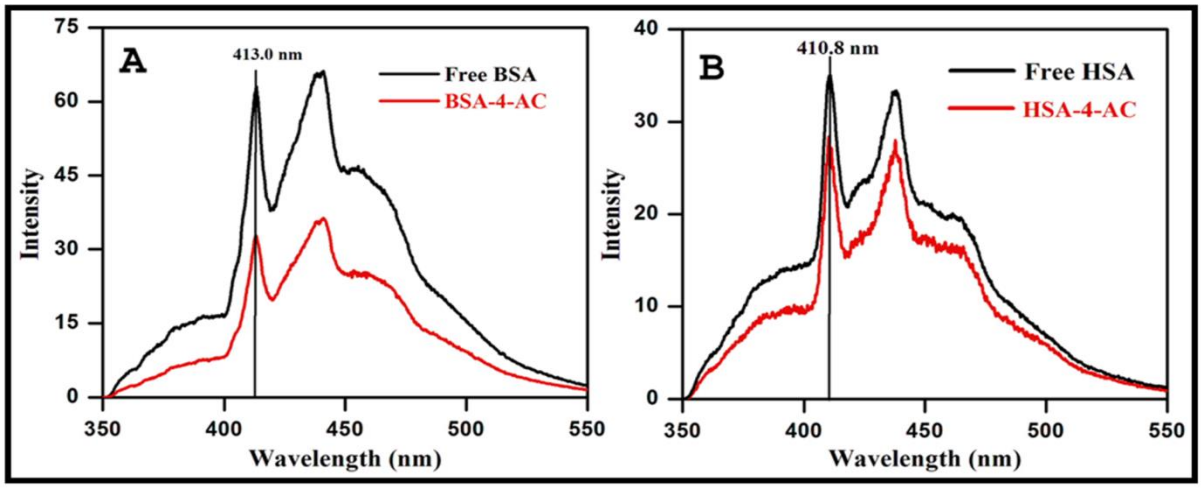


Figure 2.6. Phosphorescence spectra of (A) Free BSA and BSA-4-AC complex (B) Free HSA HSA-4-AC complex; at 77 K; λexc = 290 nm.

Table 2.2. Phosphorescence Data for serum albumins and their complexes with 4-AC in a 40% ethylene glycol Matrix at 77 K (λ_{exc} = 290 nm).

System	Position of the phosphorescence (0, 0) band (nm)[a]	width of the phosphorescence (0,0) band at half-maxima (cm^{-1})
Free BSA (10 µM)	405.6 (sh) 413.0	281
BSA-4-AC (1:1)	405.6 (sh) 413.0	257
Free HSA (10 µM)	410.8	296
HSA-4-AC (1:1)	410.2	287

Serum albumins and 4-azido coumarin enhancement study monitoring ligand emission at 298 K:

Protein ligand enhancement study is another way to understand the protein ligand interaction. This experiment is the reverse of the quenching process. The fluorescence spectra of the ligand 4-AC in aqueous buffer solution shows a band with a maxima at ~452 nm. There has been a steady increase in the emission of 4-AC with an appreciable blue shift of the emission maxima of the ligand in the presence of increasing concentration of proteins (Fig. 2.7), suggesting that the ligand molecule is experiencing a less polar or more hydrophobic environment in both the serum albumin-ligand complexes as compared to the free ligand (Fig. 2.8 and 2.9).

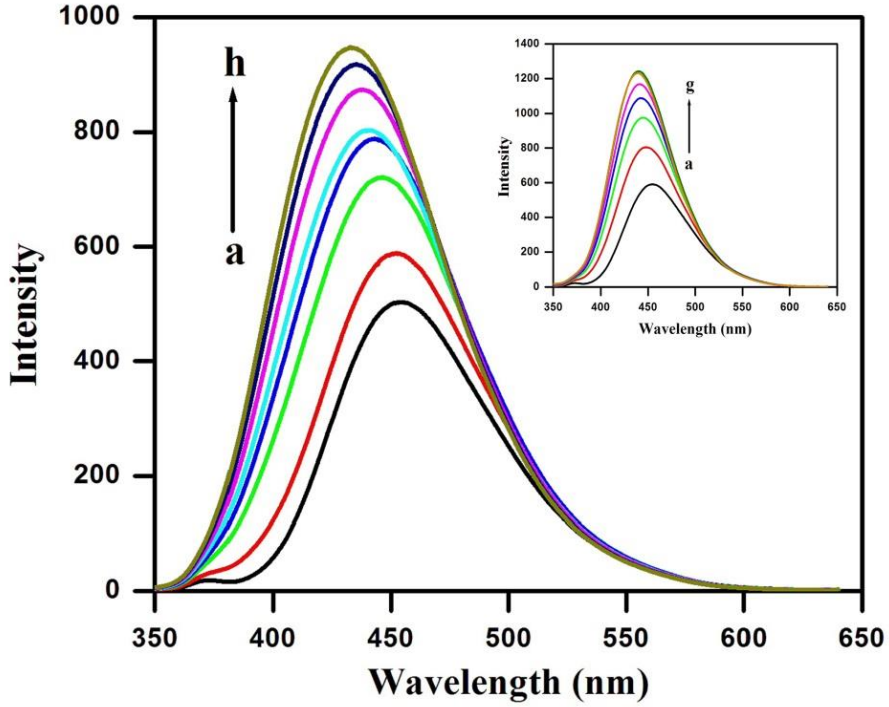


Figure 2.7. Fluorescence spectra of 4-AC with varying concentration of BSA, *Inset:* Fluorescence spectra of 4-AC with varying concentration of HSA.

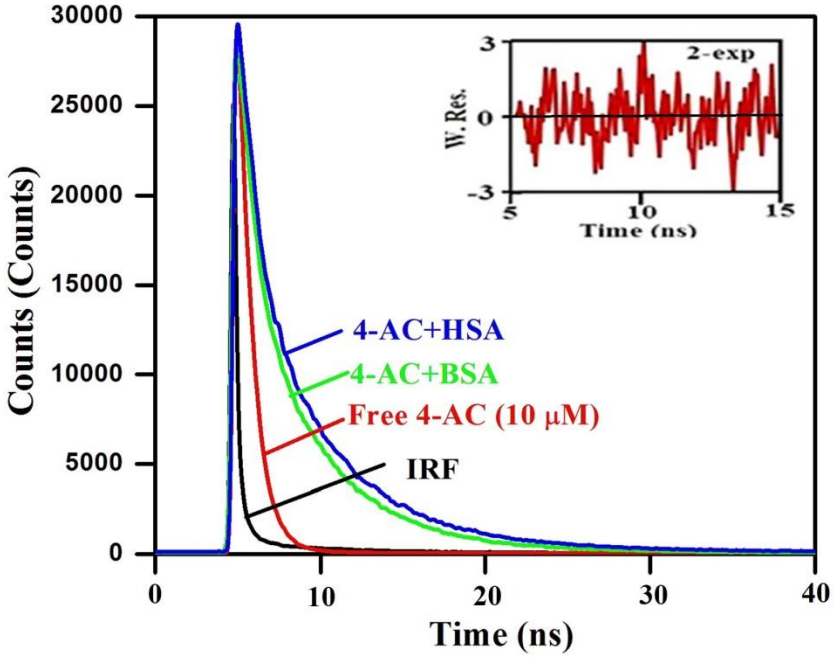


Figure 2.8. Fluorescence decay of 4-AC at 298 K in aqueous buffer (pH 7), final concentration of BSA and HSA.

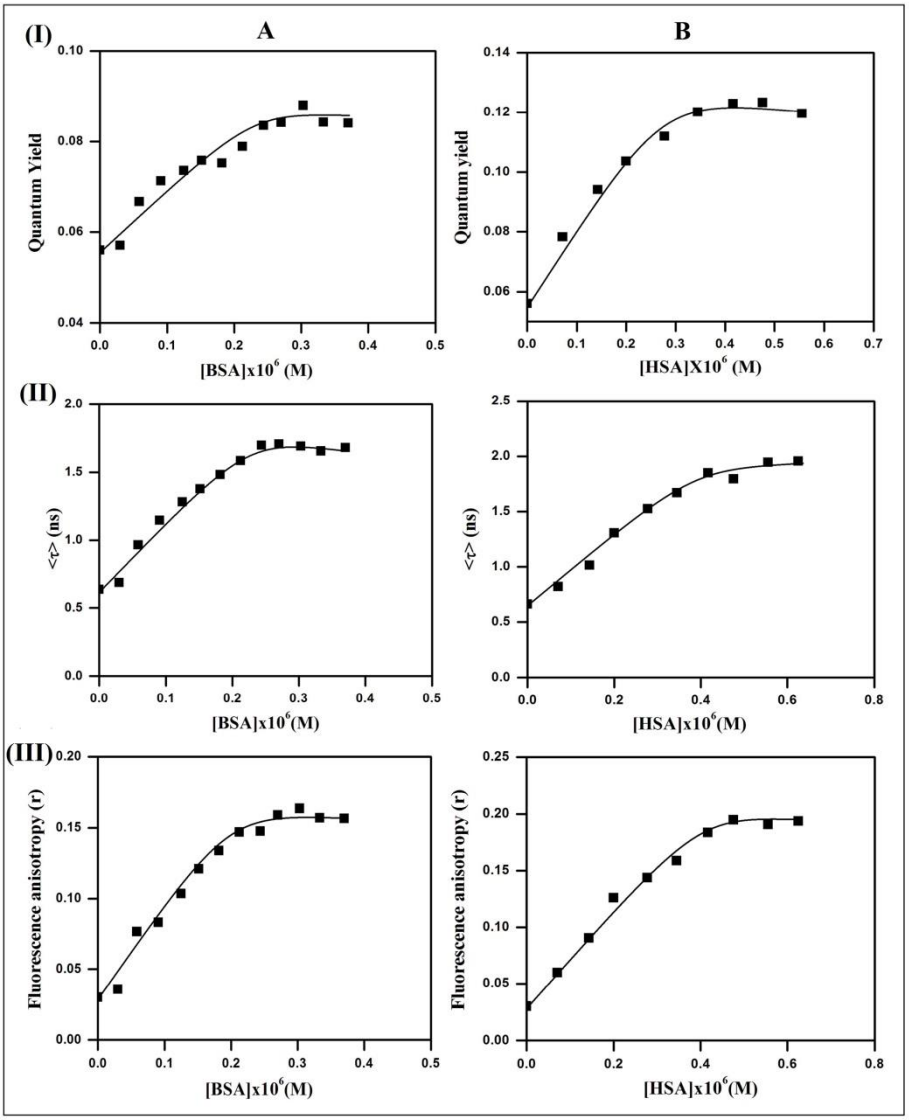


Figure 2.9. (**A**) Variation of (I) fluorescence quantum yield (Φ_F); (II) singlet state average lifetime (s); (III) fluorescence anisotropy (r)of 4-AC in aqueous buffer monitoring the enhanced emission with increasing concentration of BSA (**B**) Variation of (I) fluorescence quantum yield (Φ_F); (II) singlet state average lifetime(s); (III) fluorescence anisotropy (r)of 4-AC in aqueous buffer monitoring the enhanced emission with increasing concentration of HSA.

Chapter II B

Interaction of 4-Azido coumarin with cyclodextrins and micelles

Introduction:

Drug delivery is very important in medicinal chemistry research because sometime it has been observed that drugs are often incompatible with each other or with other ingredients present in a formulation. Encapsulation one of that incompatible ingredients within a CD molecules, stabilizes the formulation by physically separating the components in order to prevent drug-drug or drug-additive interaction.

Present work:

Present chapter discuss the interaction of 4-AC (Fig. 2.1) with cyclodextrins, α-CD, β-CD, γ-CD and micelles, C-TAB (cationic), SDS (anionic), T-60 (neutral) to understand the binding property of 4-AC with CDs and micelles, which is important to understand the drug delivery process.

Result and Discussion:

Steady state fluorescence study of 4-AC with cyclodextrins and micelles in aqueous medium 298K:

The fluorescence spectra of the ligand 4-AC in aqueous buffer solution shows an emission maxima (λ_{max}) at 452.2 nm. The fluorescence emission of free 4-AC enhanced upon addition of CDs (Fig. 2.10) and micelle (Fig 2.11) as well as blue shift. Enhancement in fluorescence emission implies that 4-AC goes into the interior cavity of both CDs and micelle and blue shift implies that 4-AC is in hydrophobic region inside the CDs and micelle compare to free 4-AC in aqueous solution (Fig. 2.12 and 2.13).

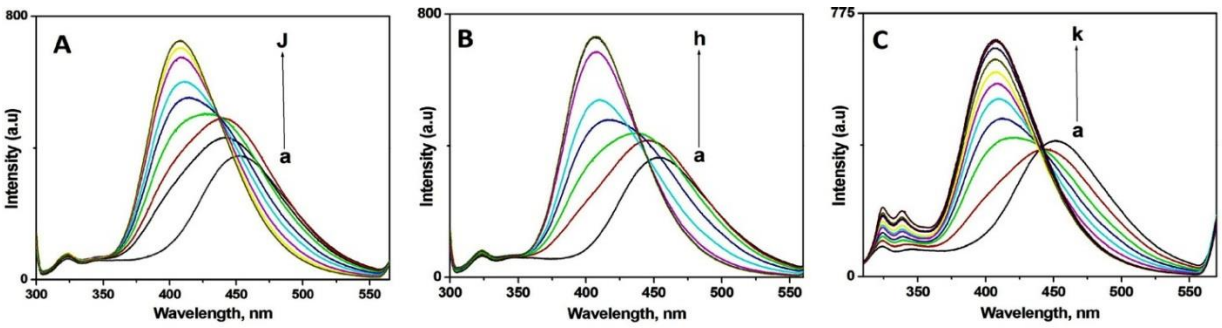


Figure 2.10. Fluorescence spectra of 4-AC with varying concentration of (A) α-CD (B) β-CD (C) γ-CD at 290nm.

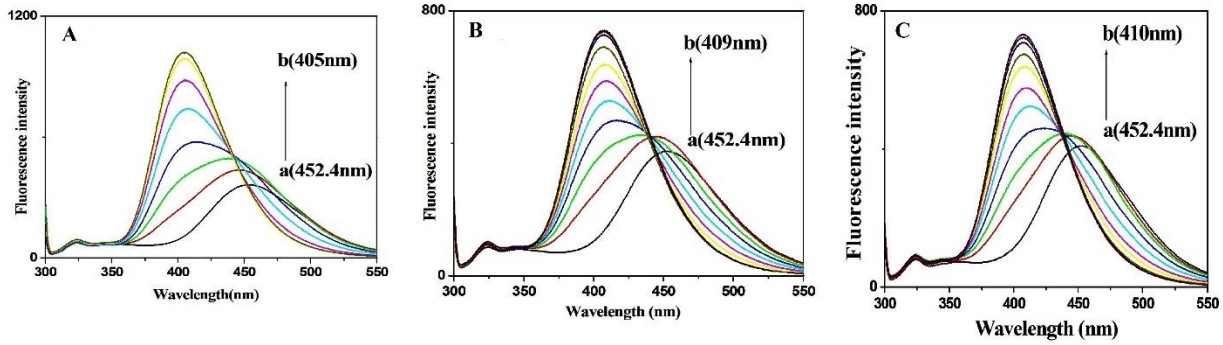


Figure 2.11. Fluorescence spectra of 4-AC with varying concentration of (A) C-TAB (B) T-60 (C)SDS at 290nm.

Fluorescence enhancement studies of 4-AC-cyclodextrins complexes:

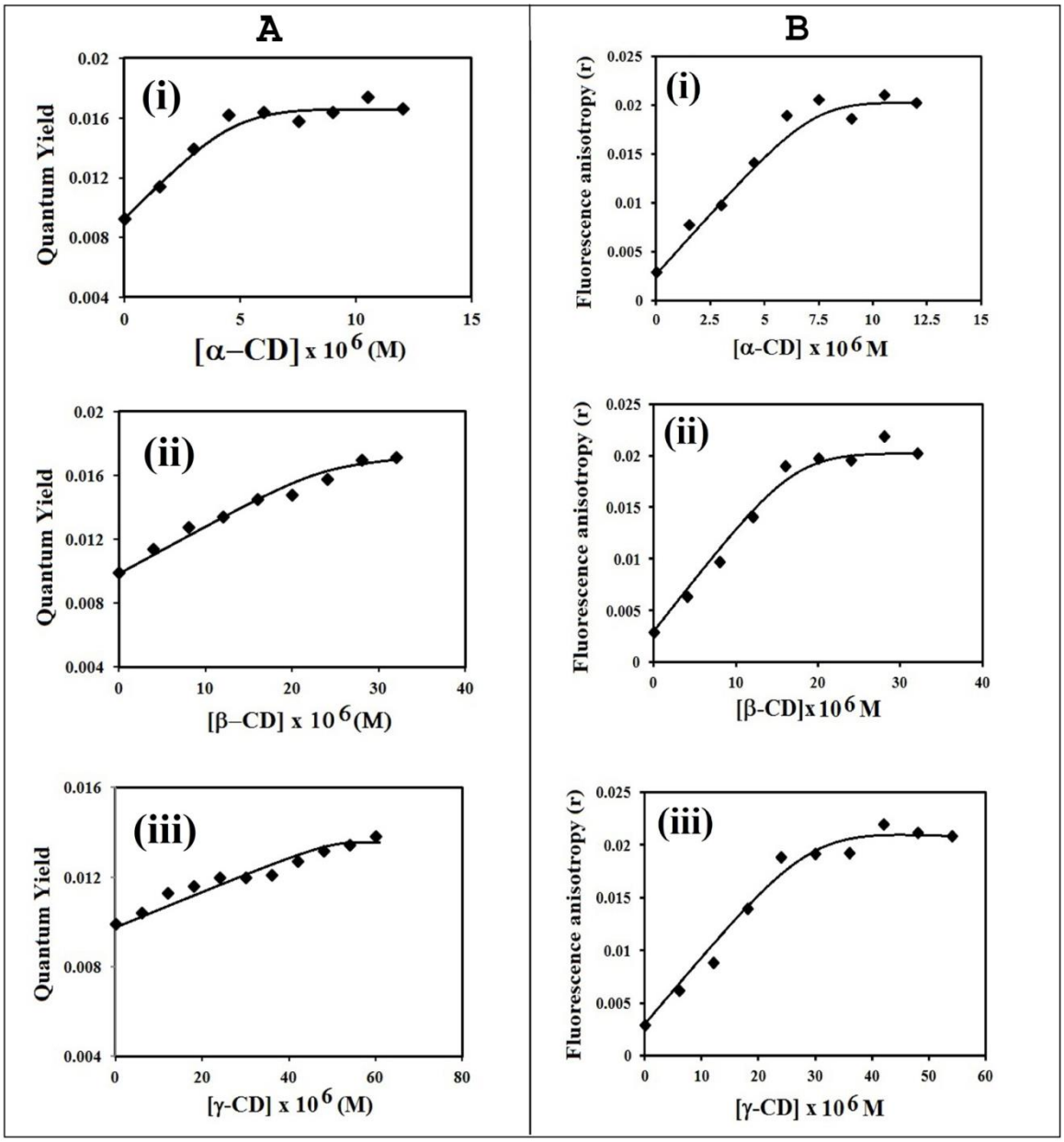


Figure 2.12. Variation of (A) fluorescence quantum yield (φ_F) (B) fluorescence anisotropy (r) of 4-AC (10 μM) in aqueous buffer monitoring the enhanced emission with increasing concentrations of (i) α-CD (ii) β-CD and (iii) γ-CD.

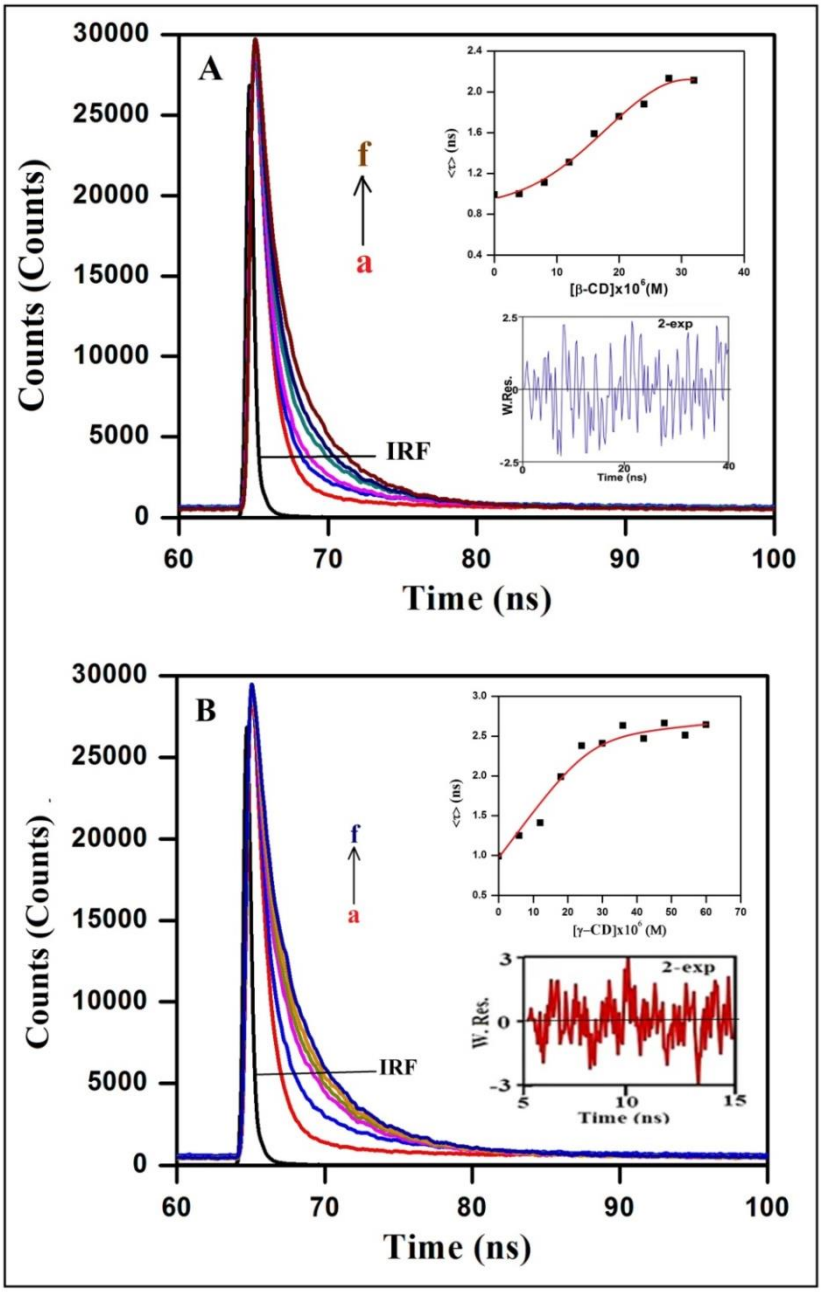


Figure 2.13. (**A**) Fluorescence decay monitoring the enhanced emission of 4-AC(10 μM) with varying concentration of β-CD, (**B**) Fluorescence decay monitoring the enhanced emission of 4-AC (10 μM) with varying concentration of γ-CD; at 298 K in aqueous phosphate buffer (pH 7).

Conclusion:

In this chapter (II-A and II-B) synthesis 4-AC was reported and then quenching and enhancement studies of 4-AC was carried out with SA using different spectroscopic technique, which indicates strong binding affinity of 4-AC towards SA. Interaction of 4-AC also been carried out with cyclodextrins and micelles. The experiment shows that 4-AC goes into the interior cavity of both CDs and micelles, thus these can act as a drug delivery carrier for 4-AC.

References:

1. D. Carter and J. X. Ho, *Adv. Protein Chem*., 1994, **45**, 153.
2. T. Kosa, T. Maruyama and M. Otagiri, *Pharm .Res*., 1997, **14**, 1607.
3. S. Paul, N. Sepay, S. Sarkar, P. Roy, S. Dasgupta, P. S. Sardar and A. Majhi, *New J. Chem*., 2017, **41**, 15392.
4. S. Paul, P. Roy, P. S. Sardar and A. Majhi, *ACS Omega*, 2019, **4**, 7213.
5. S. Krishnamoorthy, F. Xie, M. C. Brandon, L. Su, H. N.Barnhill and Q.Wang, *Org. Lett*., 2004, **4**, 4603.
6. Q. Wang and S. Krishnamoorty, International Pat., WO2005/103705A2, 2005.
7. D. P. Yeggoni, M. Gokara, D. M.Manidhar, A. Rachamallu, S. Nakka, C. S. Reddy and R. Subramanyam, *Mol. Pharmaceutics*, 2014, **11**, 1117.
8. B. Sudhamalla, M. Gokara, N. Ahalawat, D. G. Amooru and R. Subramanyam, *J. Phys. Chem. B*, 2010, **114**, 9054.
9. G. Zhang, L. Wang and J. Pan, *J. Agric. Food Chem*., 2012, **60**, 2721.
10. S. Ghosh, A. Misra, A. Ozarowski, C. Stuart and A. H. Maki, *Biochemistry*, 2001, **40**, 15024.
11. S. Ghosh, A. Misra, A. Ozarowski and A. H. Maki, *J. Phys.Chem. B*, 2003, **107**, 11520.

Publications:

[1] **Sandip Paul**, Nasim Sepay, Shrabana Sarkar, Pritam Roy, Swagata Dasgupta, Pinki Saha Sardar and Anjoy Majhi*, **New Journal of Chemistry**, **2017,** 41, 15392.

Oral presentation:

[1] **National Conference "Chemistry: Today and Tomorrow (CTT-2018)",** Interaction of 4- Azido coumarin, a fluorescent probe with biomacromolecular systems; **Sandip Paul** and Anjoy Majhi.

Poster presentation:

[1] **National Conference on Recent Developments in Nanoscience & Nanotechnology (2019).** Design and synthesis of some fluorescent probe: Studies with biomacromolecular systems; **Sandip Paul** and Anjoy Majhi.

[2] **27th West Bengal State Science & Technology Congress (2019).** Enhancement study of 4-Azido coumarin in serum albumins by spectroscopic technique; **Sandip Paul**, Pinki Saha Sardar and Anjoy Majhi.

Chapter III

Design, Synthesis, and Biophysical Studies of Thienopyridine derivatives

Introduction:

Heterocyclic compounds are important in drug development and discovery system. Thienopyrimidines are one of the interesting and important moiety for the development of drugs. In the field of medicinal chemistry so many thienopyrimidine based drugs are discovered which are now available in market. Fused pyrimidine ring present in thienopyrimidine derivative and it show interesting pharmacological and biological activities [1–3]. Thienopyrimidine show structural similarity and isoelectronic relation with purine and several substituted thieno [2,3-d] pyrimidine derivatives to exhibit their useful biological activities [4,5]. Over the last two decades so many research has been done on thienopyrimidine, which exhibit potential medicinal activities (Fig. 3.1) such as anticancer [6], analgesic [7], antimicrobial [8-9] and antiviral agents [10] and other biological activities[11-15].

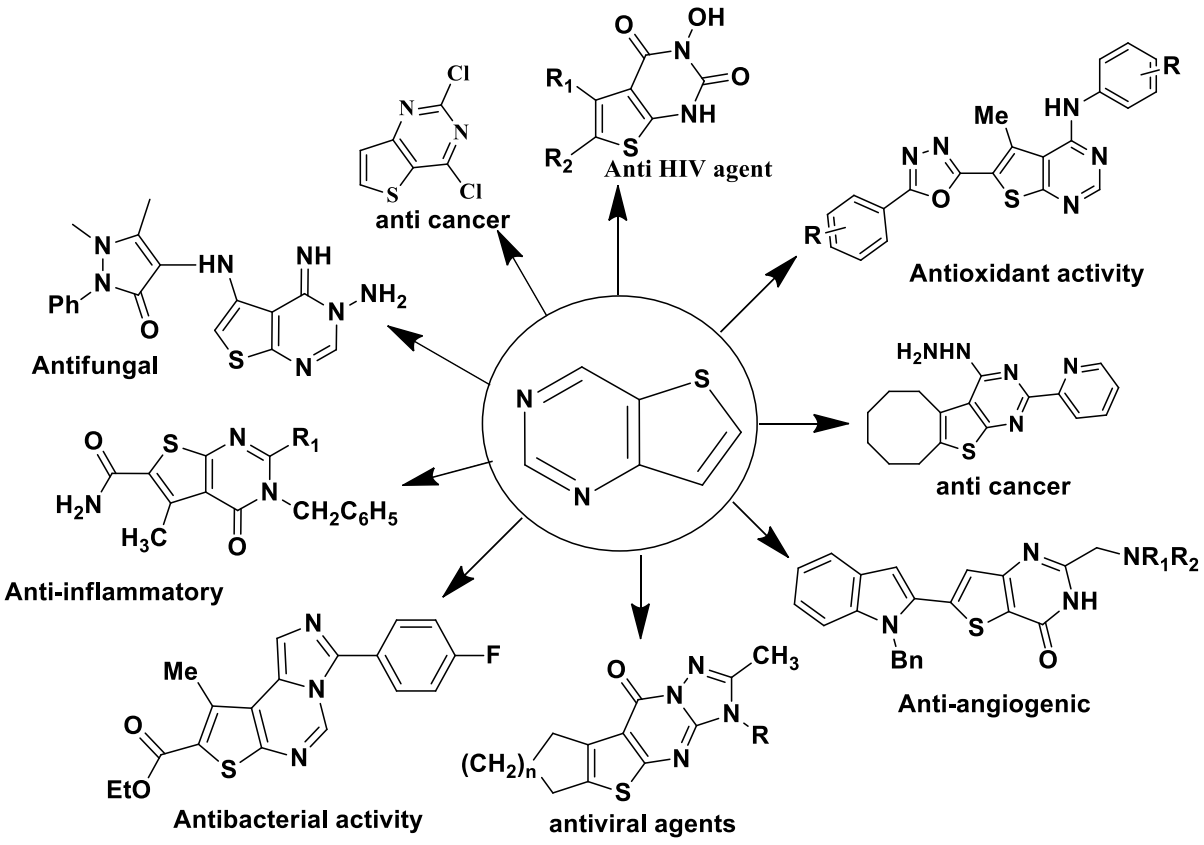


Figure 3.1. Schematic diagram of the medicinal activities of thienopyrimidine moiety

Present work:

Present work reports the design and synthesis of novel thienopyrimidine amine derivatives (Fig. 3.2) and there after photophysical interactions with bio molecules using different spectroscopic technique. Thienopyrimidine amine derivatives are capable of showing biological activities [16], so, we are focusing on the development of different thienopyrimidine amine derivatives. Interaction of the compound also been carried out with micelle to check the drug delivery process. Cytotoxicity of the compounds also been carried out in Hep G2 cell line.

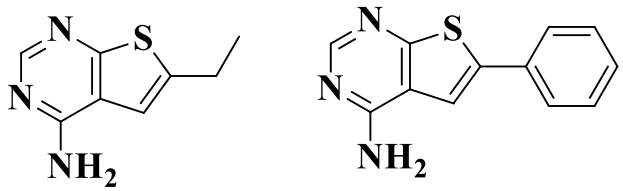
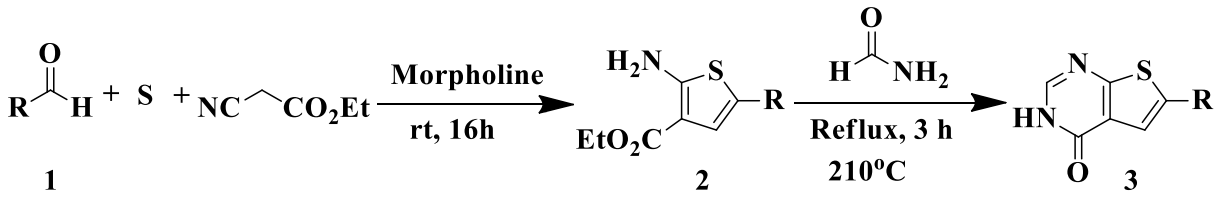


Figure 3.2. Thienopyrimidine amine derivatives

Result and Discussion:

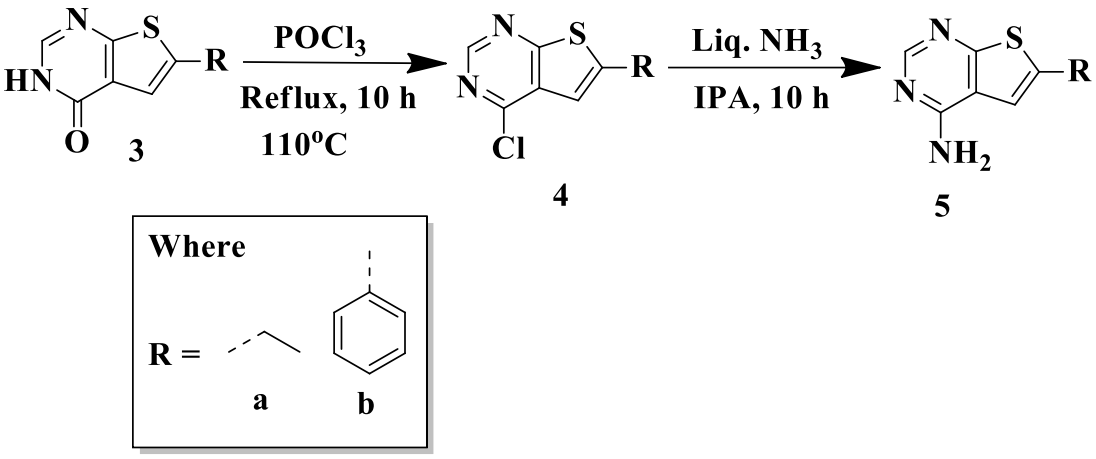
Here in this chapter we have discussed the synthesis, characterization and photophysical studies of compound **5a** and **5b**. Compound **5a** does not shows promising interactions either with biomolecules (Serum albumins) and supra molecules (C-TAB), however compound **5b** shows promising interactions with biomolecules as well as supra molecules. Cytotoxicity assay has also been carried out in this chapter for the compound **5a** and **5b**.

Scheme 3.1. Methodology for the synthesis of compound 3:



Procedure: Mixture of aldehyde, sulphur powder, ethyl cyanoacetate and morpholine was stirred at room temperature for 16 hours, which affords compound **2**. Then compound **2** was refluxed with formamide at 210^{o}C for 3 hours to get compound **3** (Scheme 3.1).

Scheme 3.2. Methodology for the synthesis of compounds 5 (a and **b):**



Procedure: Compound **5** was synthesized according to the procedure described in scheme 3.2. Compound **3** was refluxed with phosphorus oxychloride ($POCl_3$) at 110°C for 10 hours, affords compound **4**. Finally compound **4** was treated with liq. NH_3 in isopropyl alcohol (IPA) to obtained desired product compound **5**.

Characterisation data of **5a: ^{1}H NMR (400 MHZ, ($CDCl_3$)**, δ: 1.161 (3H, t, J= 6 Hz), 2.698 (2H, q), 6.973 (1H, s), 7.732 (1H, s); **^{13}C NMR (400 MHZ, ($CDCl_3$)**, δ: 15.36, 24.15, 117.09, 125.17, 142.93, 146.63, 159.67, 164.37; **MS-ESI (+):** $C_8H_{10}N_3S$ $[M+H]^+$ Found 180.97.

Characterisation data of **5b: ^{1}H NMR (400 MHZ, ($CDCl_3$)**, δ: 7.425 (1H, S), 7.464 (1H, dd, J= 4 Hz, 2 Hz), 7.533 (2H,d, J= 2.4 Hz), 7.769 (2H, d, J=7.2 Hz), 8.138 (2H, S), 9.5 (1H, S); **^{13}C NMR(400 MHZ, ($CDCl_3$)**, δ: 116.80, 126.41, 129, 129.37, 133.28, 142.69, 143.15, 158.67, 164.51; **MS-ESI (+)**: $C_{12}H_{10}N_3S$ $[M+H]^+$ Found 228.08.

Absorption, emission and binding study of compound 5b with proteins at 298 K:

The interaction of compound **5b** with proteins (BSA and HSA) and micelle (C-TAB) shows that the absorption spectra of compound **5b** slightly changed upon addition of increasing concentration of proteins and micelle (Fig. 3.3), this is the preliminary indication of protein ligand interaction.

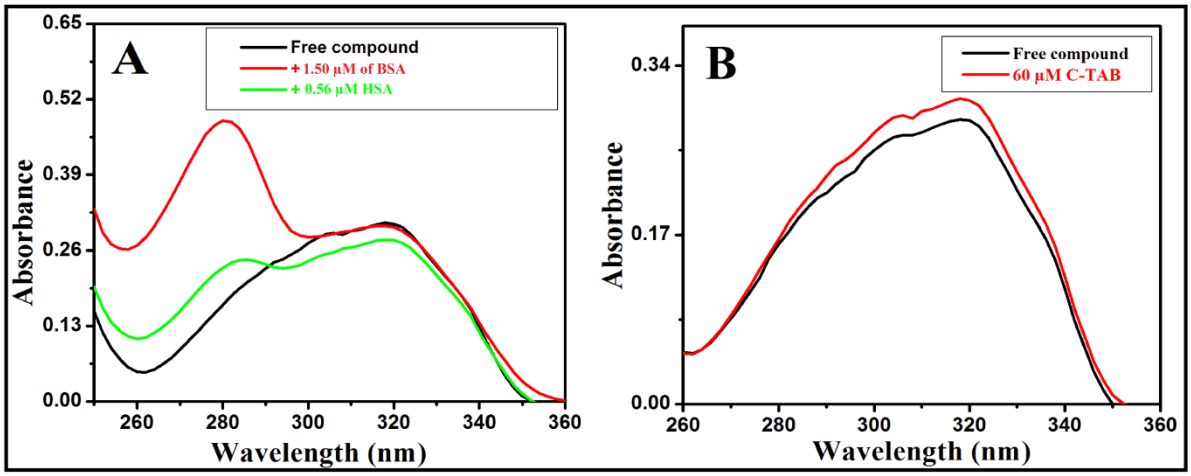


Figure 3.3. Absorption spectra of compound **5b** and (**A**) varying concentration of BSA and HSA (**B**) varying concentration of C-TAB in aqueous phosphate buffer (pH 7) at 298 K.

The fluorescence emission spectroscopy of **5b** shows an enhancement upon addition of increasing concentration of proteins and C-TAB (Fig. 3.4). The increase of fluorescence intensity in presence of proteins and micelle indicates the incorporation of the ligand **5b** within a less polar (hydrophobic) protein pocket (Fig. 3.5). In hydrophobic environment, non radiative decay channels are largely blocked, and this leads to the enhancement in fluorescence intensity [17] (Fig. 3.6, Table 3.1).

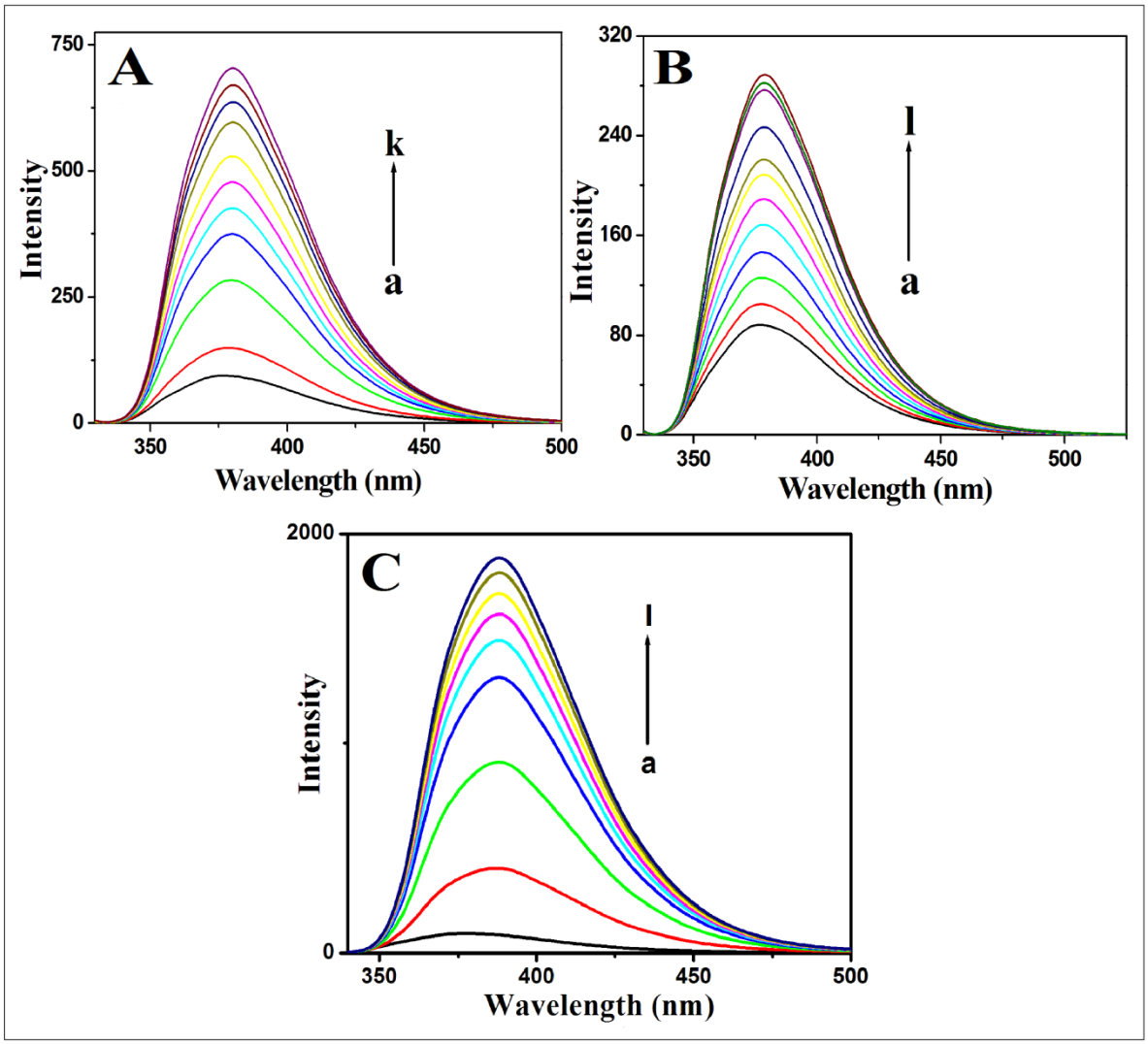


Figure 3.4. Fluorescence spectra of compound **5b** and **(A)** varying concentration of BSA **(B)** varying concentration of HSA **(C)** varying concentration of C-TAB in aqueous phosphate buffer (pH 7) at 298 K.

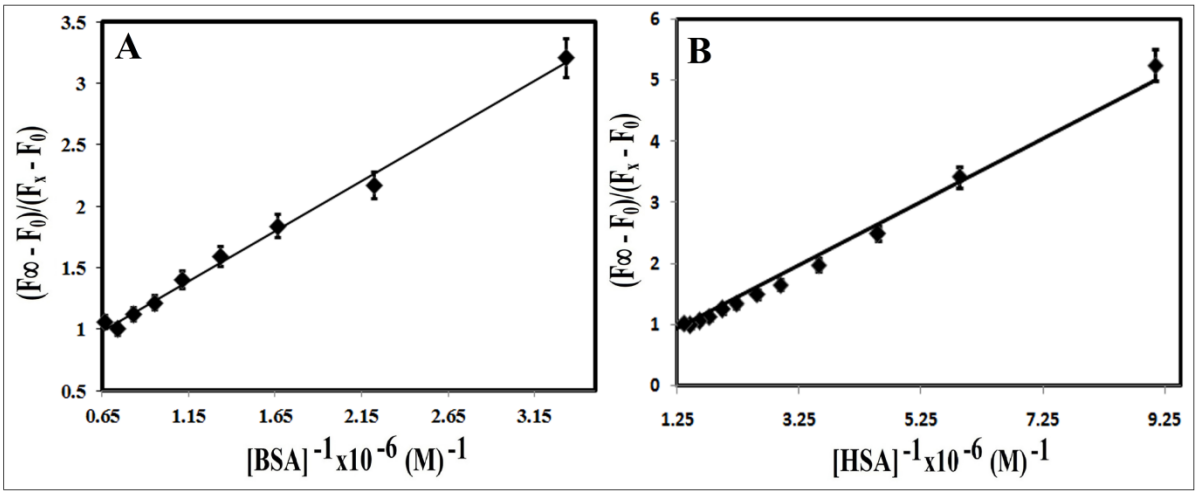


Figure 3.5. The plot of (**A**) $[F_\infty - F_0]/[F_x - F_0]$ against $[BSA]^{-1}$, and (**B**) $[F_\infty - F_0]/[F_x - F_0]$ against $[HSA]^{-1}$.

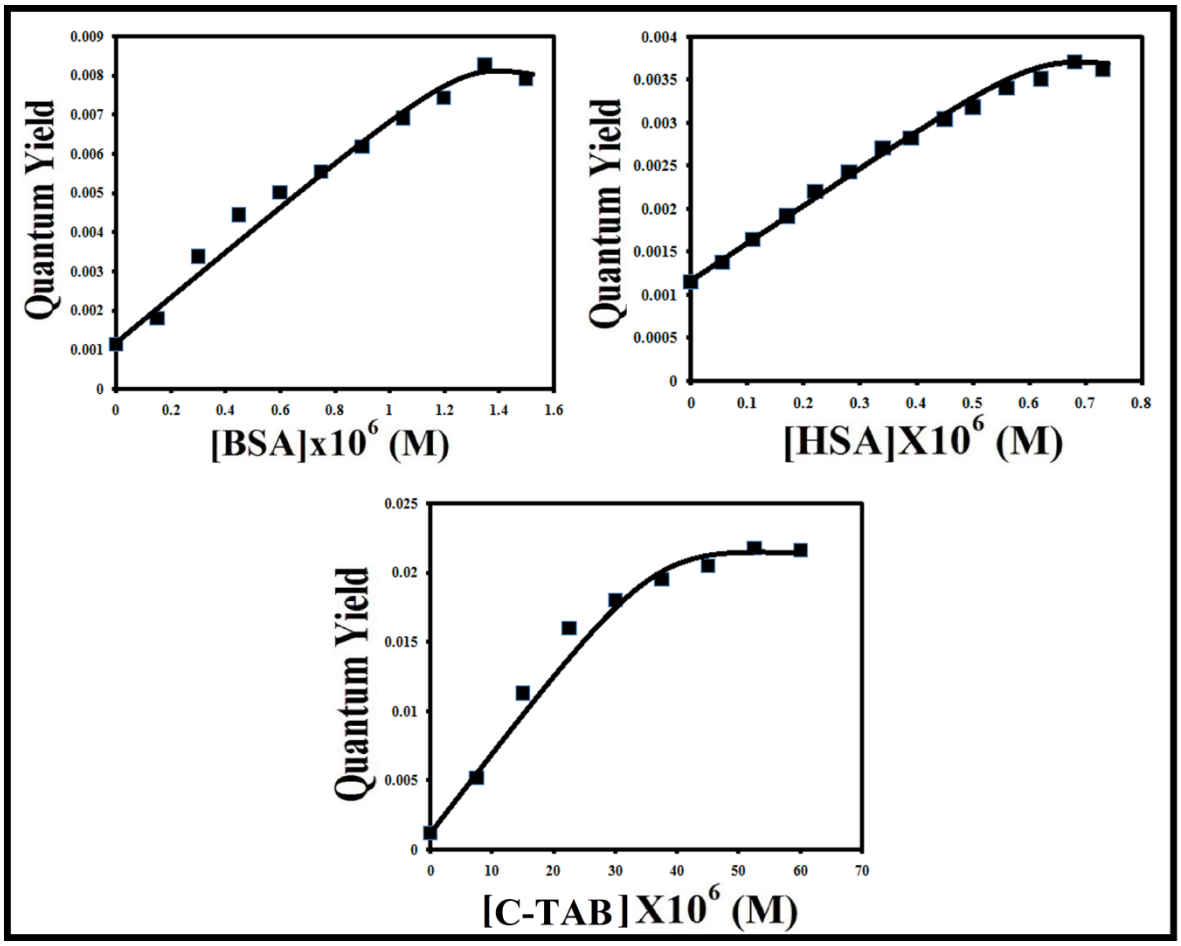


Figure 3.6. Fluorescence Quantum yield of compound **5b** and **(A)** varying concentration of BSA **(B)** varying concentration of HSA **(C)** varying concentration of C-TAB in aqueous phosphate buffer (pH 7) at 298 K.

Table 3.1. Radiative, nonradiative decay and binding constant value of compound **5b** and its complex with BSA, HSA and C-TAB

System	Kr × 10^6 (S^{-1})	Knr × 10^6 (S^{-1})	Binding Constants (K) M^{-1}
Compound **5b**	0.0002	1.049	-
BSA- **5b** Complex	0.0005	0.52	1.89 x10^6
HSA- **5b** Complex	0.0005	0.81	2.70 x10^6
C-TAB - **5b** Complex	0.05	0.48	0.13x10^6

Cytotoxicity Assay:

The cytotoxicity of the compounds **5a** and **5b** has been studied through MTT assay in Hep G2 cell line (Fig. 3.7). Cytotoxicity and IC50 values have been determined using standard procedure. Compounds **5a** and **5b** show moderate cytotoxicity at a concentration of 50 μM, which gradually increase upon increasing concentration of the compounds and finally at a concentration of 200 μM. Compounds **5a** and **5b** show cytotoxicity at higher concentration (200 μM).

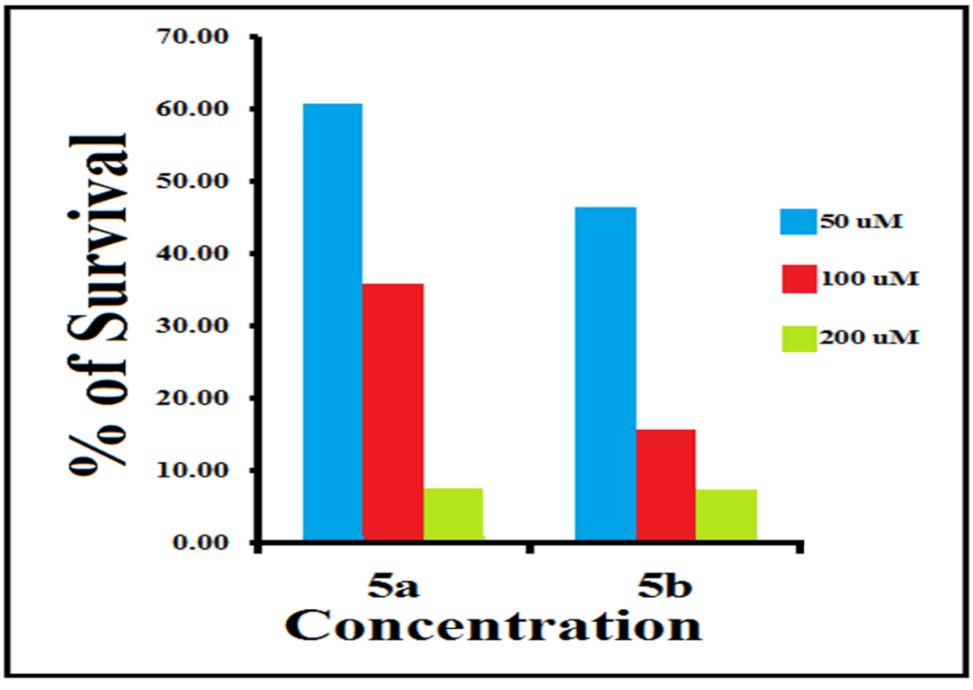


Figure 3.7. % Cell viabilityof Hep G2 cells at different concentrations (50–200μM) of compounds 5a-b.

Conclusion:

Here in this chapter, synthesis of novel thienopyrimidine derivatives was reported and then photophysical and biological studies of the synthesize compounds have been carried out. Drug delivery process also been checked and it shows promising results with micelle (C-TAB). Cytotoxicity assay of the compounds were carried out in Hep G2 cell line, which shows cytotoxicity at higher concentration (200 μM), so, it may not be suitable for used as a drug and further studies can provide better insight for the development drugs in the future.

References:

1) Y. A. Ibrahim and A. H. M. Elwahy, *Adv. Heterocycl. Chem.*, 1996, **65**, 235.
2) K. A. Ismail, O. M. Aboulwafa and E. Koreish, *Farmaco*, 1995, **50**, 611.
3) A. G. Hammam, M. Sharaf and N. A. Abdelhafez, *Indian J. Chem.*, 2001, **40B**, 213.
4) A. E. Rashad, A. H. Shamroukh, R. E. A. Megeid and W. A. E. Sayed, *Synth. Commun*., 2010, **40**, 1149.
5) A. E. Rashad, A. H. Shamroukha, H. H. Sayed, S. M. Awad and A. M. Abdelwahed, *Synth. Commun.*, 2011, **41**, 652.
6) A. E. Amr, A. M. Mohamed, S. F. Mohamed, N. A. A. Hafez and A. G. Hammam, *Bioorg. Med. Chem*., 2006, **14**, 5481.
7) A. E. Amr, M. I. Hegab, A. A. Ibrahim and M. M. Abdalah, *Monatsh. Chem*., 2003, **134**, 1395.
8) N. A. Hassan, M. I. Hegab, A. E. Rashad, A. A. Fahmy and F. M. A. Megeid, *Nucleosides, Nucleotides*, 2007, **26**, 379.
9) K. M. Elmahdy, A. M. Elkazak, M. A. Megid, M. Seada and F. Osama, *J. Advances in Chem*., 2013, **5**, 581.
10) A. E. Rashad and M. A. Ali, *Nucleosides, Nucleotides*, 2006, **25**, 17.
11) W. Wang, D. Lv, N. Qiu, L. Zhang, C. Hu, and Y. Hu, *Bioorg. Med. Chem. Lett*., 2013, **21**, 2886.
12) E. Perpicace, V. J. Hureaux, R. Ragno, F. Ballante, S. Sartini, C. L. Motta, F. D. Settimo, B. Chen, G. Kirsch, S. Scheider, B. Faivre, and S. Hesse, *Eur. J. Med. Chem*., 2013, **63**, 765.
13) S. I. Panchamukhi, A. K. M. Iqbal, A. Y. Khan, M. B. Kalashetti, and I. M. Khazi, *Pharm. Chem. J*., 2011, **44**, 694.

14) D. Liuyu, G. Houying, Z. Qiuming, H. Xinming, and G. Shaoyong, *Wuhan Univ. J. Nat. Sci*., 2012, **17**, 177.

15) J. Kankanala, K. A. Kirby, A. D. Huber, M. C. Casey, D. J. Wilson, S. G. Sarafianos and Z. Wang, *Eur J Med Chem.*, 2017, **141**, 149.

16) C. Y. Leung, A. M. Langille, J. Mancuso and Y. S. Tsantrizos, *Bioorg. Med. Chem.*, 2013, **21**, 2229.

17) S. Datta, S. Panja, P. Mitra and M. Halder, *Langmuir*, 2015, **31**, 10781.

Lists of Publications

[1] **Sandip Paul**, Pritam Roy, Pinki Saha Sardar and Anjoy Majhi*, **ACS Omega, 2019,** 4, 7213.

[2] **Sandip Paul**, Raju Ghanti, Pinki Saha Sardar and Anjoy Majhi*, **Chemistry of Heterocyclic Compounds, 2019,** 55(7), 607**.**

[3] **Sandip Paul**, Nasim Sepay, Shrabana Sarkar, Pritam Roy, Swagata Dasgupta, Pinki Saha Sardar and AnjoyMajhi*, **New Journal of Chemistry**, **2017,** 41, 15392.

Paper presented in National / International Symposium / Conferences

Oral presentation:

[1] **International Symposium on Recent Advances in Chemistry and Material Sciences (2019):** Design and synthesis of fluorescent probe based on HDAC scaffold and its biophysical studies; **Sandip Paul** and Anjoy Majhi.

[2] **National Conference "Chemistry: Today and Tomorrow (CTT-2018)",** Interaction of 4-Azido coumarin, a fluorescent probe with biomacromolecular systems; **Sandip Paul**, and Anjoy Majhi.

Poster presentation:

[1] **International seminar on 'ITINERARY OF CLASSICAL TO MODERN ERA OF CHEMISTRY' (2019):** Synthesis of triazole based derivatives using click reaction and their interaction with serum albumins; **Sandip Paul** and Anjoy Majhi.

[2] **National Conference on Recent Developments in Nanoscience & Nanotechnology (2019).** Design and synthesis of some fluorescent probe: Studies with biomacromolecular systems; **Sandip Paul**, and Anjoy Majhi.

[3] **27th West Bengal State Science & Technology Congress (2019).** Enhancement study of 4-Azido coumarin in serum albumins by spectroscopic technique; **Sandip Paul**, Pinki Saha Sardar and Anjoy Majhi.

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